interactions of microtubules with the cortex. J. Cell Biol. *151*, 1337–1344.
5. Bloom, K. (2001). Nuclear migration: cortical anchors for cytoplasmic dynein. Curr. Biol. *11*, R326–R329.
6. Evangelista, M., Zigmond, S., and Boone, C. (2003). Formins: signaling effectors for assembly and polarization of actin filaments. J. Cell Sci. *116*, 2603–2611.
7. Dimmer, K.S., Fritz, S., Fuchs, F., Messerschmitt, M., Weinbach, N., Neupert, W., and Westermann, B. (2002). Genetic basis of mitochondrial function and morphology in Saccharomyces cerevisiae. Mol. Biol. Cell *13*, 847–853.
8. Fehrenbacher, K.L., Boldogh, I.R., and Pon, L.A. (2005). A role for Jsn1p in recruiting the Arp2/3 complex to mitochondria in budding yeast. Mol. Biol. Cell *16*, 5094–5102.
9. Boldogh, I.R., and Pon, L.A. (2006). Interactions of mitochondria with the actin cytoskeleton. Biochim. Biophys. Acta *1763*, 450–462.
10. Hoppins, S., Lackner, L., and Nunnari, J. (2007). The machines that divide and fuse Mitochondria. Annu. Rev. Biochem., epub ahead of print.
11. Griffin, E.E., Graumann, J., and Chan, D.C. (2005). The WD40 protein Caf4p is a component of the mitochondrial fission machinery and recruits Dnm1p to mitochondria. J. Cell Biol. *170*, 237–248.
12. Schauss, A.C., Bewersdorf, J., and Jakobs, S. (2006). Fis1p and Caf4p, but not Mdv1p, determine the polar localization of Dnm1p clusters on the mitochondrial surface. J. Cell Sci. *119*, 3098–3106.
13. Roux, A., Uyhazi, K., Frost, A., and De Camilli, P. (2006). GTP-dependent twisting of dynamin implicates constriction and tension in membrane fission. Nature *441*, 528–531.
14. Legesse-Miller, A., Massol, R.H., and Kirchhausen, T. (2003). Constriction and dnm1p recruitment are distinct processes in mitochondrial fission. Mol. Biol. Cell *14*, 1953–1963.
15. Fehrenbacher, K.L., Yang, H.C., Gay, A.C., Huckaba, T.M., and Pon, L.A. (2004). Live cell imaging of mitochondrial movement along actin cables in budding yeast. Curr. Biol. *14*, 1996–2004.
16. Taguchi, N., Ishihara, N., Jofuku, A., Oka, T., and Mihara, K. (2007). Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. J. Biol. Chem. *282*, 11521–11529.
17. De Vos, K.J., Allan, V.J., Grierson, A.J., and Sheetz, M.P. (2005). Mitochondrial function and actin regulate dynamin-related protein 1-dependent mitochondrial fission. Curr. Biol. *15*, 678–683.

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Evolutionary Genomics: A Dinosaur's View of Genome-Size Evolution

Estimates of cell volume in fossilized bones of extinct dinosaurs indicate that genome size underwent a significant reduction in the early theropods, from which birds later evolved. This suggests that birds' small genomes are not an adaptation to metabolic demands associated with flight.

Hans Ellegren

Is the small genome size uniformly seen among extant birds an adaptation to the energetic demands of flight? This question has puzzled geneticists for quite some time, not just because of an urge to understand avian genome evolution. More generally, we may ask whether genome size is a selected character that readily responds to changes in life history and life style. Or does variation in genome size merely reflect neutral mutational processes like the success of 'selfish' DNA elements, which have no or only limited effects on an organism's fitness?

Eukaryotic genomes show an impressive range of size variation which, in contrast to early views, does not correlate closely with organismal complexity, posing the famous 'C-value paradox'. For instance, there are many examples of both animals and plants that have genomes larger (~3 Gb) than the human genome by an order of magnitude or more. The genome sizes of tetrapods (amniotes and amphibians) vary from ~1 Gb to over 100 Gb, with the highest values seen in salamanders (>50 Gb), amphiumas (>75), waterdogs (120) and lungfish (130). Genome size correlates negatively with metabolic rate in poikilothermal as well as homeothermal amniotes [1], and it has been hypothesized that a high metabolic rate requires a relatively small genome size [2]. The postulated explanation lies in the positive correlation between genome size and cell size [3]: because smaller cells have a larger surface-to-volume ratio, their rate of gas exchange per unit volume is higher than for larger cells. The final link in this chain of observations and arguments is the so-called nucleoskeletal theory, which proposes a co-evolutionary link between cell size and genome size, such that more DNA causes nuclei and cells to swell.

The observations that birds and bats have the smallest genomes among vertebrates, and that, among birds, flightless species tend to have the largest genomes, led to the idea that the metabolically intense demands of powered flight introduced a constraint on genome size [4,5]. According to this view, the evolution of avian flight was accompanied by a reduction in size and streamlining of genomes. While interesting, the hypothesis has remained speculative, because it has not been possible to test if there was a transition from large to small genomes when the early ancestors of modern birds diverged from other dinosaurs (birds, Aves, are now recognised as being nested within theropod dinosaurs, which are bipedal predators). However, Organ *et al.* [6] have now brought genomics to the extinct world of dinosaurs by capitalising on the correlation between genome size and cell size. They estimated the size of osteocytes (bone cells) in 31 extinct species of dinosaurs by measuring the cavities in fossilized bone in which cells once resided. After having calibrated the relationship between osteocyte size and genome size using data from extant species, dinosaur genome sizes were then

extrapolated by a Bayesian comparative method.

The new analyses indicate that small genomes were present already in the first theropods, including *Tyrannosaurus rex*, that evolved 230 million years ago and also in a single sample of a sauropod (sister group to theropods) that evolved approximately 250 million years ago (Figure 1). However, species from the other primary clade of dinosaurs, ornithischians, mainly herbivores, had larger genomes, comparable in size to those of non-avian reptiles such as lizards, geckos and alligators. These variations in genome size most likely reflect differences in the lineage-specific activities of mobile interspersed repeat elements [7], which have been low in birds [8] and probably also in other theropod dinosaurs [6]. Another recent study shows that the repetitive landscape of non-avian reptiles by and large is similar to that of mammals [9].

According to these observations, small genomes appeared long before the first birds entered the scene about 150 million years ago. As a consequence, a reduction in genome size does not seem to have been the result of an adaptation to the metabolic costs associated with flight. Yet Organ *et al.* [6] speculate that there might be an adaptive explanation to genome reduction in the early dinosaurs, because of the physiological demands associated with maintaining a high body temperature.

The approach taken in the study of Organ *et al.* [6] is novel and the results are convincing and have broad significance. But what about the conjecture that a small genome size in theropod/sauropod dinosaurs evolved in response to the physiological demands associated with endothermy? Is it realistic to think of natural selection being strong enough for organisms to evolve mechanisms that lead to shrinkage of genomes, or at least prevent genomes from further expansion? This is questionable when it comes to size changes introduced by small chromosomal insertions and deletions which often involve only one or a few

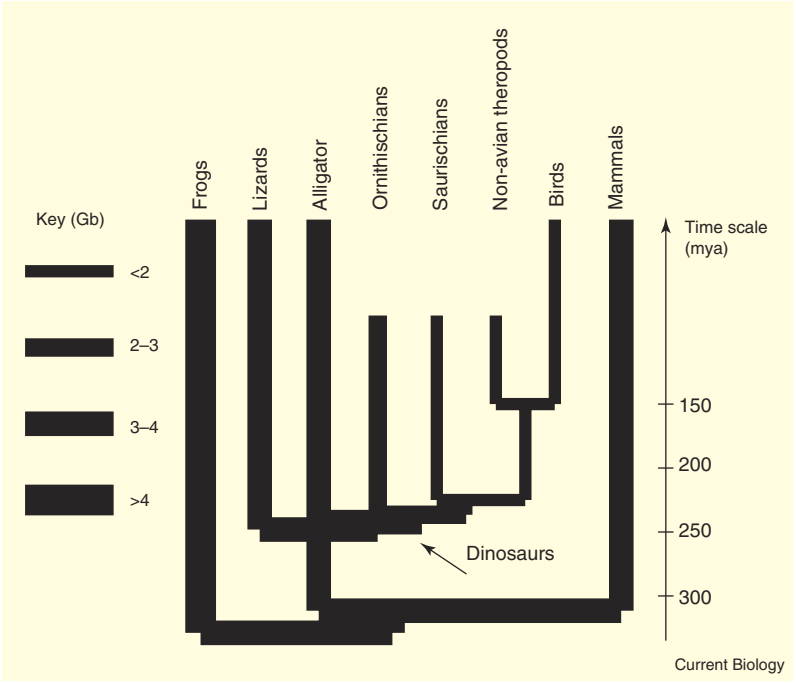

Figure 1. Schematic illustration of genome size evolution in vertebrate groups.

nucleotides at a time [10]; it is difficult to imagine a situation in which the fitness of two individuals differs due to an infinitesimal difference in genome size caused by such minute length mutations. Expansion of families of retroelements would have larger effects on genome size, but there is still the question of whether individual fitness would be significantly affected by within-population differences in copy number of interspersed repeats. That the insertion of retroelements in coding sequence, and likely also in non-coding regulatory sequence, can have dramatic effects on fitness is well established; however, this should apply broadly to genomes irrespective of other adaptations.

The determinants of genome size are likely to be several and their relative importance continues to be a hotly debated issue [11–16]. The selectionist view of evolution of genome size in response to energetic and metabolic adaptation is attractive, but a causal relationship remains to be demonstrated. One alternative scenario that needs to be considered is that the fixation probability of slightly deleterious mutations — in this case represented by retroelement insertions — is not only determined by their selective disadvantage but also by the effective population size; the larger the population the smaller the chance for a mildly deleterious mutation to drift to fixation. It is noted that, on the broad scale from prokaryotes to unicellular eukaryotes to multicellular eukaryotes, a reduction in population size is accompanied by an expansion in genome size [17]. Nonadaptively, and according to the nearly neutral theory of molecular evolution, this can be taken as support for the idea that purifying selection becomes less efficient at removing proliferating repeat elements as populations become smaller. But whether this model is applicable to bird and dinosaur evolution is unclear. It would be interesting to see the results of simulations designed to test the feasibility of the model.

Another issue that calls for attention in the context of adaptive genome size evolution is the relationship between genome organisation and genome size. Avian genomes are not only small, they are also characterised by containing numerous and minute microchromosomes, dot-like chromosomes that are densely

packed with genes, have short introns and little repetitive DNA [18]. Microchromosomes are also seen in some but not all non-avian reptiles [19] so it is not a derived character specific to birds. But could it be that selection has favoured the maintenance of this form of genome organisation in the avian lineage as part of possible constraints on genome size? It would be terribly exciting to learn about chromosomal structure in non-avian dinosaurs but the outstanding question at present is: how?

References

1. Vinogradov, A.E., and Anatskaya, O.V. (2006). Genome size and metabolic intensity on tetrapods: a tale of two lines. Proc. R. Soc. Lond. B *273*, 27–32.
2. Cavalier-Smith, T. (1995). The Evolution of Genome Size (Chichester: Wiley).
3. Gregory, T.R. (2001). Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol. Rev. *76*, 65–101.
4. Tiersch, T.R., and Wachtel, S.S. (1991). On the evolution of genome size in birds. J. Hered. *82*, 363–368.
5. Hughes, A.L., and Hughes, M.K. (1995). Small genomes for better fliers. Nature *377*, 391.
6. Organ, C.L., Shedlock, A.M., Meade, A., Pagel, M., and Edwards, S.V. (2007). Origin of avian genome size and structure in non-avian dinosaurs. Nature *446*, 180–184.
7. Kazazian, H.H., Jr. (2004). Mobile elements: Drivers of genome evolution. Science *303*, 1626–1632.
8. International Chicken Genome Sequencing Consortium (ICGSC) (2004). Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature *432*, 695–716.
9. Shedlock, A.M., Botka, C.W., Zhao, S., Shetty, J., Zhang, T., Liu, J.S., Deschavanne, P.J., and Edwards, S.V. (2007). Phylogenomics of nonavian reptiles and the structure of the ancestral amniote genome. Proc. Natl. Acad. Sci. USA *104*, 2767–2772.
10. Hartl, D.L. (2000). Molecular melodies in high and low C. Nat. Rev. Genet. *1*, 145–149.
11. Petrov, D.A. (2001). Evolution of genome size: new approaches to an old problem. Trends Genet. *17*, 23–28.
12. Petrov, D.A. (2002). Mutational equilibrium model of genome size evolution. Theor. Pop. Biol. *61*, 533–546.
13. Gregory, T.R. (2002). A bird eye's view of the C-value enigma: genome size, cell size, and metabolic rate in the class Aves. Evolution *56*, 121–130.
14. Vinogradov, A.E. (2004). Evolution of genome size: multilevel selection, mutation bias or dynamical chaos? Curr. Opin. Genet. Dev. *14*, 620–626.
15. Gregory, T.R. (2004). Insertion-deletion biases and the evolution of genome size. Gene *324*, 15–34.
16. Gregory, T.R. (2005). Synergy between sequence and size in large-scale genomics. Nat. Rev. Genet. *6*, 699–708.
17. Lynch, M., and Conery, J.S. (2003). The origins of genome complexity. Science *302*, 1401–1404.
18. Ellegren, H. (2005). The avian genome uncovered. Trends Ecol. Evol. *20*, 180–186.
19. Burt, D.W. (2002). Origin and evolution of avian microchromosomes. Cytogenet. Genome Res. *96*, 97–112.

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Membrane Protein Chaperones: A New Twist in the Tail?

The integration of tail-anchored membrane proteins at the endoplasmic reticulum occurs via a specialised ATP-dependent pathway, but the cytosolic factors involved have proven elusive. A novel ATPase that mediates this process has now been identified.

Catherine Rabu and Stephen High

Tail-anchored proteins are a specialised class of integral membrane proteins that display the bulk of their polypeptide chain to the cytosol and play key roles in numerous cellular processes including vesicular transport, protein translocation and apoptosis. These proteins are characterised by a carboxy-terminal hydrophobic transmembrane domain, the location of which dictates that their membrane integration must occur post-translationally (Figure 1). Whilst tail-anchored proteins are inserted into several different eukaryotic organelles, many studies have focused on their membrane integration at the endoplasmic reticulum (ER), from where they can also reach locations throughout the secretory pathway [1,2]. From the outset it was apparent that the targeting and integration of tail-anchored proteins was distinct from the classical co-translational pathway [3]. Hence, early studies showed that the integration of the tail-anchored protein cytochrome b_5 was quite unusual and did not rely on the signal recognition particle (SRP) [4].

A major development in the field arose from the study of another archetypal tail-anchored protein, synaptobrevin 2, providing definitive proof that the post-translational integration of tail-anchored proteins requires ATP [5]. This study also developed the use of short carboxy-terminal extensions, in this case incorporating a site for N-glycosylation, to provide unambiguous evidence that tail-anchored proteins are correctly integrated into the membrane [5] (Figure 1). A role for ATP [6–9], and in some cases GTP [9,10], for the efficient post-translational insertion of several different tail-anchored proteins is now well established (Figure 1). Furthermore, there is good evidence that the ATP dependency reflects a role for molecular chaperones [7,9]. Until now, the identity of these putative chaperones has been unclear and attention has focused on likely candidates such as Hsc70 [9]. A recent paper now suggests that a completely new component plays a key role in mediating the ATP-dependent delivery of many tail-anchored proteins to the ER [11].

Stefanovic and Hegde [11] used the β-subunit of the Sec61 translocon as their model tail-anchored protein and, using both cross-linking and co-immunoprecipitation analyses, identified a strong post-translational interaction with a 40 kDa protein present in the reticulocyte lysate that is typically